# MELISSA

## ESTEC/CONTRACT 8125/88/NL/FG

## Technical note 10

# TN 10 PHOTOAUTOTROPHIC GROWTH OF RHODOSPIRILLUM RUBRUM AND RHODOBACTER CAPSULATA.

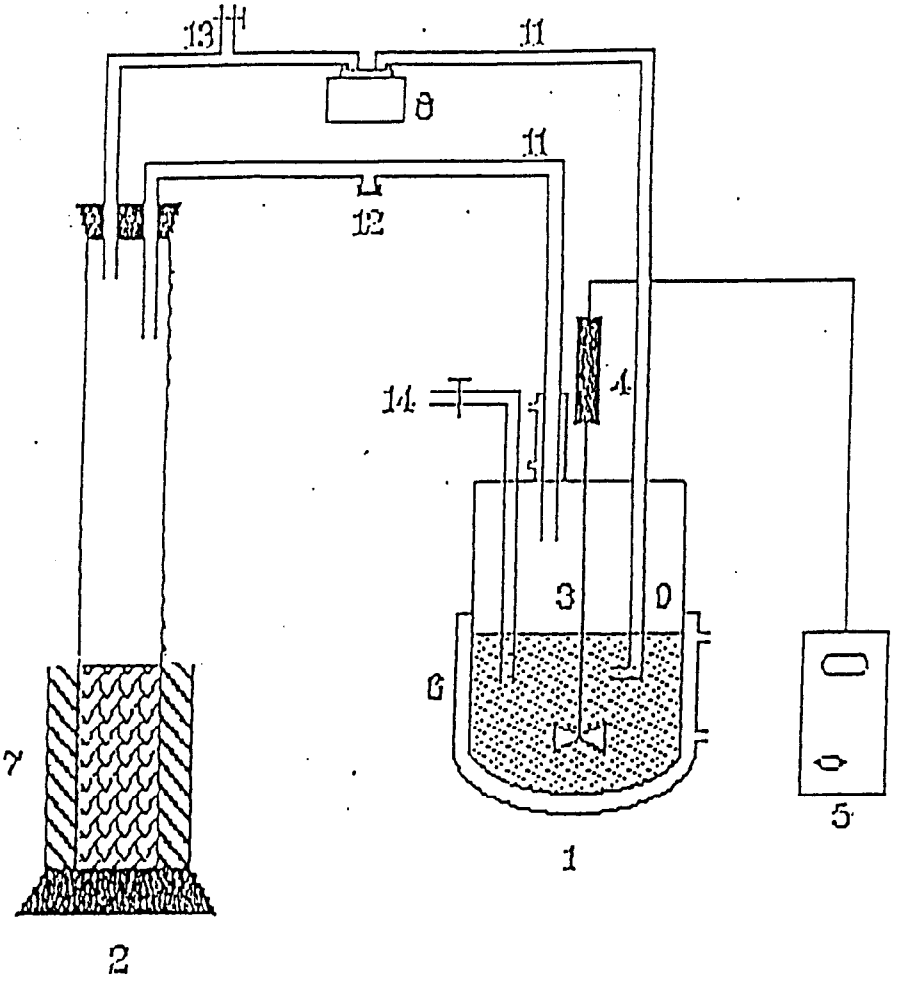
## 1. Introduction.

Purple non-sulphur bacteria are capable of photoheterotrophic growth on an array of organic carbon sources and some have also been reported as being able to grow photoautotrophically with $H_2$ as an electron donor and $CO_2$ reduction (Yoch, 1978). It was therefore suggested to split the second compartment of the Melissa cycle into an autotrophic and an heterotrophic compartment, handling respectively the gas phase and the soluble effluent from the liquefying compartment. The division is necessary because $H_2$ consumption is inhibited by organic compounds. These organic compounds are to be metabolized completely in the heterotrophic compartment, in order to have an $NH_4^+$-rich effluent. Indeed, ammonium can be used as nitrogen source for the fotoautotrophic compartment as well as for the nitrification compartment.

## 2. Materials and Methods.

*Strains.*
*Rhodobacter capsulata* LMG5162
*Rhodospirillum rubrum* LMG4362$^T$

*Reactor.*
A reactor for autotrophic growth was set up (Fig.1 and Fig.2). A gas mixture of 20% $CO_2$ as carbon source and 80% $H_2$ as inorganic electron source was bubbled through the basal salts medium (Segers and Verstraete, 1983) with 0.5 g/l $(NH_4)_2SO_4$ as nitrogen source. The reactor was illuminated with a Sylvania Gro-Lux Fluorescent Lamp F40/T12. The light intensity in the middle of the empty reactor vessel was 150 $E.m^{-2}.s^{-1}$(as measured with a Li-Cor Li 185




1. Reactor
2. Gas column
3. Stirrer
4. Stirrer motor
5. Speed control
6. Double wall of reactor vessel
7. Buffer pH=3
8. Air pump
9. Gas inlet
11. Gas recirculation
12. Gas sampling point
13. Gas inlet
14. Sampling point

Fig. 1 : Diagrammatic representation of reactor vessel for photoautotrophic growth.

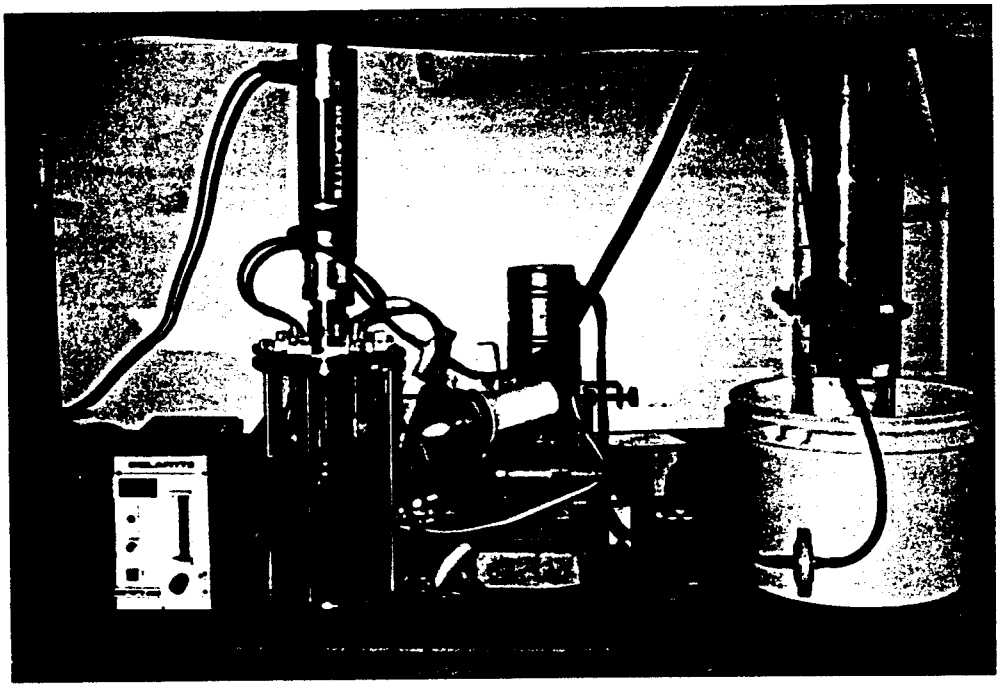


Fig. 2 Reactor vessel for photoautotrophic growth.

B quantumsensor). The incubation temperature was maintained at 30°C by passing water through the double wall of the reactor.

*Analyses.*

The ammonium-nitrogen content of the medium was determined by steam distillation in a Kjeltec.1002 apparatus. The protein content of the biomass was determined by the Bradford (1976) method.

**3. Results.**

Both organisms, *Rhodospirillum rubrum* and *Rhodobacter capsulata*, were capable of photoautotrophic growth (Fig.3 and Fig.4). The ammonium nitrogen was assimilated far more rapidly by *Rh. capsulata* than by *R. rubrum*. The amount of gas utilized by *R. rubrum* could not be measured as there was a slight leak in the system. This was noticed as the nitrogen concentration of the gas phase increased from 0 to 21% after one week. *R. rubrum* may have used the dinitrogen as additional N-source.

*Rh. capsulata* produced 0.7 g/l protein biomass after 160 hours (6.5 days) i.e. 1.2 g dry weight/l. *R. rubrum* produced 0.54 g/l protein biomass after 170 hours i.e. 0.9 g dry weight/l.

Table 1 summarizes the major characteristics of the photoautotrophic growth.

Table 1 : Autotrophic growth characteristics of *Rh. capsulata* and *R. rubrum*.

| Strain | Incubation time (h) | Biomass protein Total (g/l) | Biomass protein Prod. rate (g/l.d) | $NH_4^+$-assimilation rate (mg $NH_4^+$-N/l.d) |
|---|---|---|---|---|
| *Rh. capsulata* | 160 | 0.70 | 0.105 | 40 |
| *R. rubrum* | 200 | 0.65 | 0.078 | 11 |

**4. Discussion.**

Results show that the non-sulfur purple bacteria grow well under autotrophic conditions. However, photoautotrophic growth requires both anaerobic conditions and the absence of any organic C or N source. The latter condition implements a complete mineralisation of the organic substances in the liquefaction compartment and the photoheterotrophic compartment. Therefore, further research should emphasize on the optimisation of the C and N flux through these compartments.

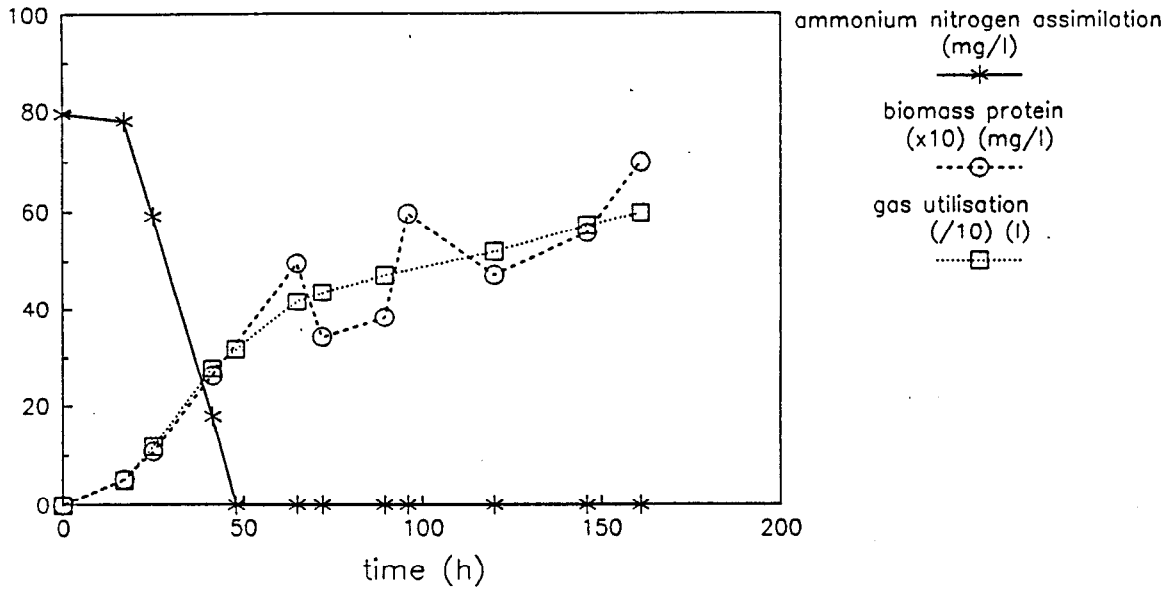



Fig. 3 :Photoautotrophic growth of *Rh. capsulata.*

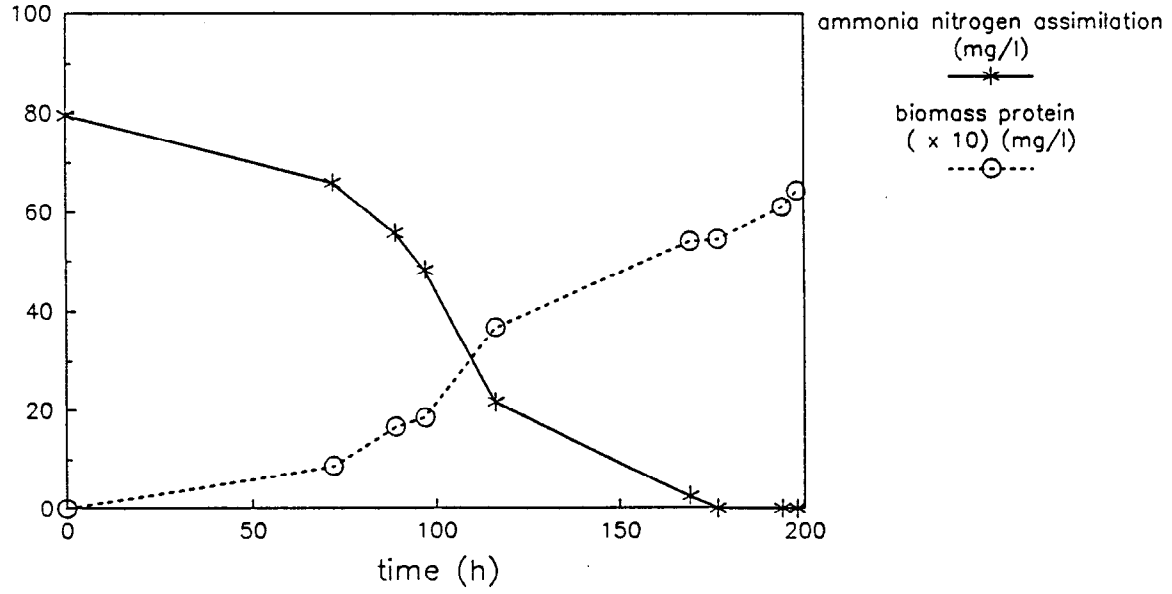



Fig. 4 :Photoautotrophic growth of *R. rubrum.*

## 5. References.

Bradford M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72: 248-254.

Segers L. and Verstraete W. (1983). Conversion of organic acids to $H_2$ by *Rhodospirillaceae* grown with glutamate or dinitrogen as nitrogen source. Biotechnol. Bioeng. 25: 2843-2853.

Yoch D. (1978). Nitrogen fixation and hydrogen metabolism by photosynthetic bacteria. In: Clayton R. and Sistron W. (Eds.) The fotosynthetic bacteria, Plenum press, New York, pp. 657-676.